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First record of *Parasola lilatincta* from Pakistan

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Abstract—*Parasola lilatincta* a lilaceous coprinoid mushroom (*Psathyrellaceae, Agaricales*) is characterized and illustrated from Malakand, Pakistan. Morpho-anatomical description and sequencing of the ITS region of the rDNA are given. This is a new record for Pakistan.

Key words—*Basidiomycota,* phylogeny, plicate pileus, taxonomy

Introduction

Parasola Redhead et al. was introduced when the coprinoid genus *Coprinus* Pers. was divided into four genera: *Coprinopsis* P. Karst., *Coprinellus* P. Karst., *Parasola* and *Coprinus* s.s. (Redhead et al. 2001). *Coprinus* s.s. was retained in *Agaricaceae*, while the other three taxa were placed in the newly validated family *Psathyrellaceae*. *Parasola* species have small and deeply plicate pilei, ranging from yellow-brown to tawny and paler colors. *Parasola* is morphologically separated from the closely related *Coprinellus*, *Coprinopsis* and *Psathyrella* (Fr.) Quél. by the complete absence of a veil structure and the lack of pileo- and caulocystidia (although *P. conopilus* (Fr.) Örstadius & E. Larss. and *P. auricoma* (Pat.) Redhead et al. do possess sclerocystidia; Nagy et al. 2010, Schafer 2014).

Index Fungorum (www.indexfungorum.org) lists 22 species under *Parasola*, although other taxonomic treatments recognize a lower number (Nagy et al. 2009, 2010; Schäfer 2010, 2014). These mushrooms, which are common decomposers of leaf-litter, wood, and herbivore dung, are distributed worldwide, with most of the records from Europe, North America, South America,

Australasia, and Africa (Pegler 1966, 1983; Grgurinovic 1997; Pennycook 2004; Schafer 2014) and some from Asia (Ahmad 1980; Pegler 1986).

The lilaceous coloration reported for the pileus in *P. lilatincta* (Uljé & Bender 1997) is not always found (Nagy 2007). The phylogeny of *Parasola* and other coprinoid fungi suggest that morphological variations like spore size, shape and position of germ pore, and presence/absence of lilaceous granules in the tissues are species delimiting characters (Nagy et al. 2009, 2012, 2013).

Only three *Parasola* species (*P. auricoma* (Pat.) Redhead et al., *P. plicatilis* (Curtis: Fr.) Redhead et al. and *P. setulosa* (Berk. & Broome) Redhead et al. have been reported previously from Pakistan (Ahmad 1980), all morphologically identified. In this study we characterized a fourth species for Pakistan using morphological and phylogenetic criteria: *P. lilatincta.* Our work also offers the first phylogenetic confirmation of *Parasola* from the country.

Materials & methods

Sampling

Samples were collected in August of 2013 and 2014 from district Malakand, KP, Pakistan (34°33–56'N 71°52–55'E), which features a subtropical climate with heavy annual rainfall (600–1100 mm) and high temperatures during monsoon (Karim 2008).

Basidiomata were found on leaf-litter, photographed, and tagged. Macroscopic features like color, shape, and size of pileus and stipe and gill attachment were noted in the field. Colors were designated according to Munsell (1975). Air-dried samples were stored in zip-lock bags prior to anatomical and molecular characterization in the lab. Specimens are conserved in the Herbarium, Hazara University, Dhodial, Mansehra, Pakistan (HUP).

Microscopical examination

Slides were prepared in 5% aqueous KOH (w/v) and lactic acid. Basidiospore size and shape, basidia, cheilocystidia, and germ pore position were studied under a light Meiji MX4300H microscope, and at least 20 measurements were recorded for each character, except that 48 basidiospores were measured in face view and/or side view through 100× oil immersion. Basidiospore measurements (rounded to the nearest 0.5 µm) are presented as follows: length range × breadth range × width range. Q values were calculated as: Q_1 = length divided by breadth; Q_2 = length divided by width (Nagy et al. 2010). Average length, breadth, and width of all basidiospores are given by avX. Line drawings were made with camera lucida.

DNA extraction, PCR amplification, and DNA sequencing

For DNA extraction, a small piece (20–40 mg) of gill was kept in 2% CTAB buffer, following Bruns (1995) with some modification. The ITS-nrDNA region was amplified using the universal primer pair ITS-1F and ITS-4 (White et al. 1990). The polymerase chain reaction (PCR) was performed in 20 µL reaction volume containing 1 µL genomic DNA, 0.1 µL of each primer in 100 pmol/µL concentration, 10 µL MasterMix (Sigma-Aldrich Co. LLC), and 8.8 µL deionized distilled water according to Gardes & Bruns

(1993) with PCR parameters set at one cycle at 94 °C for 1 min, 35 cycles at 94 °C, 53 °C and 72 °C each for 1 min, with a final extension at 72 °C for 8 min. The amplified products were run through 1% agarose gel stained with ethidium bromide and visualized under UV light Gel Documentation System (UVtec, Avebury House, Cambridge CB4 1QB UK), using default settings. The PCR products were directly sequenced in both directions using the same primer set through Automatic Sequencer 3730XL (Macrogen Korea). ITS sequences generated from this study as well as other sequences retrieved from GenBank were aligned through ClustalW followed by manual adjustment in BioEdit (www.mbio.ncsu.edu/bioedit/bioedit.html) to construct DNA dataset. Phylogeny based on 30 ITS sequences (including outgroup *Coprinus comatus* AY635772) was

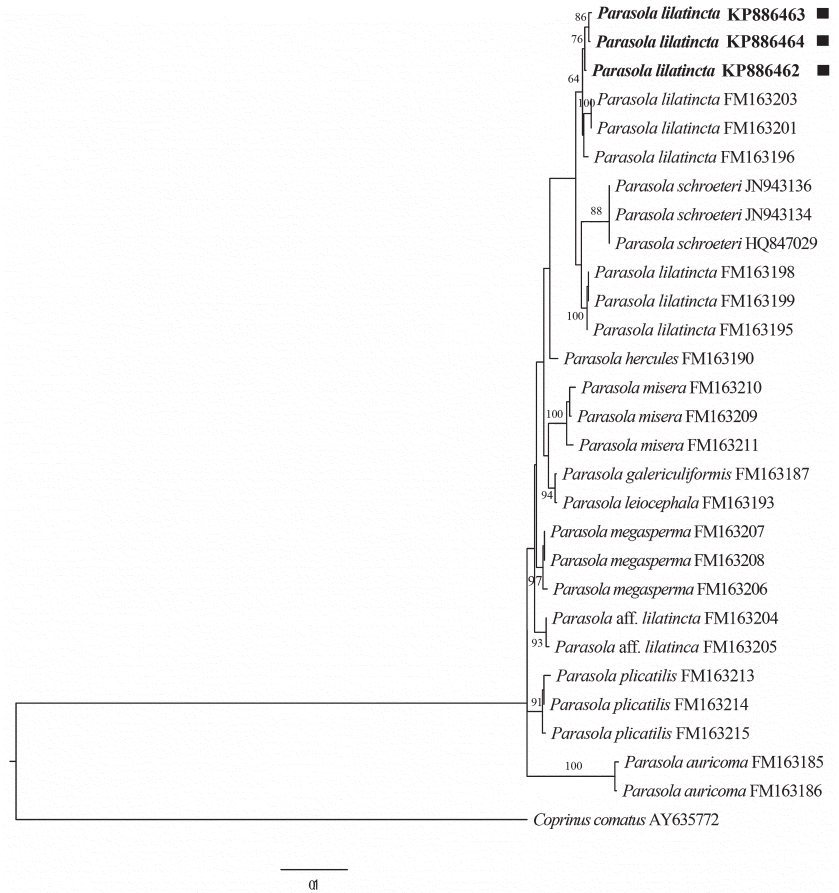

Fig. 1: ITS phylogeny of *Parasola* sequences, including Pakistani sequences of *P. lilatincta* with boldface represented by (■), inferred by the maximum likelihood method. Bootstrap support is indicated for selected nodes.

analyzed by Maximum Likelihood (ML). Phylogenetic tree was constructed through the RAxML-HPC BlackBox (8.1.11) with default setting via CIPRES Science Gateway (Miller et al. 2010).

Results

Phylogenetic analysis

Three ITS sequences of *Parasola lilatincta* were generated for this study (Fig. 1); other sequences (including the outgroup) were retrieved from GenBank. Our phylogenetic analysis closely associates the Pakistani sequences with other *P. lilatincta* sequences, but the clade structure was not well supported.

Taxonomy

Parasola lilatincta (Bender & Uljé) Redhead, Vilgalys & Hopple, Taxon 50: 236. 2001. Plates 1, 2

Pileus 15–20 mm, broadly convex to applanate, deeply plicate and membranous and smooth, light grayish brown (7.5YR 5/2–6/2); center slightly depressed, light orange (7.5R 5/2); sulcate straight margin, lacking any velar structure. Gills free, even, distant, initially concolorous with the pileus, later blackish, and finally collapsed. Stipe 40–70 × 2–4 mm, equal, central, hollow; surface smooth, snow-white.

Basidiospores [48/4/3]; (12.4)13.5–15(16.4) × (10)10.5–11(11.7) × (8.1)8.5–9.5(10.2) µm, [av*X* = 14.4 × 10.8 × 9.2; Q1 = 1.3–1.4, Q2 = 1.3–1.5, avQ = 1.4], in face view lentiform to rounded triangular, in side view ellipsoid; dark brown, germ pore 1.7–2.7 µm and eccentric, wall ≤2 µm thick. Basidia 17–23 × 6–9 µm, clavate to cylindrical, di- to tetrasporic, light brown, smooth. Cheilocystidia 25–32 × 6–9 µm, cylindrical, hyaline. Pileipellis 28–34 × 9–12 µm, clavate, hyaline, smooth with greenish oil droplets. Pleurocystidia absent.

Specimens examined: **PAKISTAN**, **Khyber Pakhtunkhwa**, Malakand, Qaldara, ca. 510 m, on leaf litter, 3 Aug. 2013, Shah Hussain (HUP–SHP2; GenBank KP886463); 10 Aug. 2014, Shah Hussain (HUP–SHP4; GenBank KP886462); 14 Aug. 2014, Shah Hussain (HUP–SHP9; GenBank KP886464).

Comments

In this study we describe *P. lilatincta* morpho-anatomically and phylogenetically as a new record for Pakistan. Proper identification of *Parasola* species is often difficult due to minor anatomical differences separating them. Basidiospore size and shape are the two main diagnostic characters used to distinguish species in this group (Nagy et al. 2009). Based on spore shape and germ pore position, *P. lilatincta* is a close relative of *P. leiocephala* (P.D. Orton) Redhead et al. and *P. schroeteri* (P. Karst.) Redhead et al.

Plate 1: Basidioma of *Parasola lilatincta* (HUP-SHP9). Scale bar: 10 mm.

We observed lilaceous tints in the pileipellis of different Pakistani collections. However, Nagy et al. (2010) did not find any such structures in the type material. Occurrence of oil contents in the pileipellis during this study suggest that the lilaceous tints may be a character of fresh material.

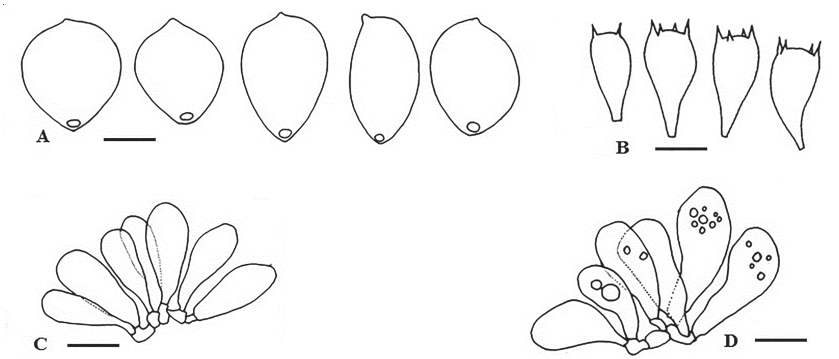

Plate 2: *Parasola lilatincta* (HUP-SHP2).
A: basidiospores; B: basidia; C: cheilocystidia; D: pileipellis elements.
Scale bars: A = 5 µm; B–D = 10 µm.

The lilaceous tints in hyphae, lentiform basidiospores, and sequence analyses all support the identification of the Pakistani collections as *P. lilatincta*.

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